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(54) Title: TUMOR TREATMENT

(57) Abstract: A method of inhibiting the growth of tumor cells. The method includes administering to a tumor site of a subject in need thereof a compound in an amount sufficient to inhibit the growth of the tumor cells at the tumor site, and, subsequently, exposing the tumor site to irradiation. The compound is a fullerene core substituted, optionally via a C_{1-50} linker, with 1-30 ionic groups.

TUMOR TREATMENT

BACKGROUND

Free radicals have been shown to inhibit tumor growth by causing oxidative damage to lipids, proteins, and nucleic acids of the tumor cells. In clinical practice, a photo-sensitizer is first delivered to a tumor site and then activated by irradiation to generate free radicals, thus inhibiting tumor growth. Among known photo-sensitizers, Photofrin II has recently been approved by the U.S. Food and Drug Administration. Preparation of Photofrin II is tedious.

Fullerenes are conjugated olefins of a closed cage structure. When photo-excited, they are capable of transforming molecular oxygen into singlet oxygen and then the related free radicals, such as superoxide free radicals, i.e., $O_2^{\cdot-}$. However, fullerenes have low bioavailability and must be chemically modified before they can be tested for their efficacy, if any, as photo-sensitizers in treating tumor.

SUMMARY

This invention relates to a method of inhibiting the growth, including causing the death, of tumor cells. The method includes administering to a tumor site of a subject in need thereof a photo-sensitive free radical-generating fullerene compound and, subsequently, exposing the tumor site to irradiation. The compound is a fullerene core which is substituted with 1-30 ionic groups, either directly or via a C_{1-50} linker. It is administered to the subject in an amount sufficient to inhibit the growth of the tumor cells at the tumor site.

The term "fullerene core" refers to C_{60}, C_{61}, C_{62}, C_{63}, C_{64}, C_{65}, C_{70}, C_{76}, C_{78}, C_{82}, C_{84}, C_{92}, La@C_{60}, La@C_{74}, La@C_{82}, Ho@C_{60}, Ho@C_{74}, Ho@C_{82}, Gd@C_{60}, Gd@C_{74}, Gd@C_{84}, Er@C_{60}, Er@C_{74}, Er@C_{82}, and the like. Among them, C_{60} is preferred.

The ionic groups mentioned above refer to those groups which are ionized in an aqueous solution at physiological pH. Examples of these ionic groups are sulfonate, sulfate, carbonate, phosphonate, phosphorate, and ammonium groups. A fullerene core, for example, can be substituted with 2-16 or 4-10 sulfonate groups to form a fullerene compound to be used in the method of this invention.

fullerene compound, e.g., $C_{60}(NH_3^+)_{12}$. Alternatively, they can be linked to the fullerene core via a C_{1-50} linker (e.g., a C_{2-16} or C_{3-8} linker). Examples of the linkers are alkyl (e.g., $-C_2H_4-$), aryl (e.g., $-C_6H_4-$), ester (e.g., $-C_3H_6CO-O-$), ether (e.g., $-C_3H_6OC_3H_6-$), thioether (e.g., $-C_7H_{14}SC_5H_{10}-$), urethane (e.g., $-C_4H_8NH-CO-O-$), urea (e.g., -NH-CO-NH-), amide (e.g., -CO-NH-), anhydride (e.g., -CO-O-CO-), amine (e.g., $-NHC_2H_4-$), and ketoether (e.g., $-CO-C_3H_6-O-C_5H_{10}-$). The fullerene core, for example, can be substituted with 6 sulfonate groups, each via a $-C_4H_8-$ linker, to form $C_{60}(-C_4H_8-SO_3^-)_6$.

The fullerene compounds described above include their pharmaceutically acceptable salts. Such salts can be formed between a negatively charged ionic group (e.g., sulfonate or carbonate) and a positively charged counterion. Suitable counterions include, but are not limited to, sodium, potassium, calcium, or magnesium. Likewise, a positively charged ionic group (e.g., ammonium) can form a salt with an anion (e.g., chloride, bromide, or iodide). One example of the salts that can be used to practice the method of this invention is hexa(sulfobutyl)fullerene sodium.

A fullerene compound to be used to practice the method of this invention is formulated into a pharmaceutical composition prior to its use in tumor treatment. Thus, also within the scope of the invention is the composition which contains such a fullerene compound and a pharmaceutically acceptable carrier for use in treating the tumor. Examples of the carriers include water, colloidal silica oxide, magnesium sterate, lipid, lipoprotein, blood protein, or cellulose. The invention also relates to use of the just-described fullerene compound for the manufacture of a medicament for the treatment of a tumor.

One of the above-described fullerene compounds, as an active ingredient in a pharmaceutical composition, is first administered to a tumor site of a subject before the tumor site is exposed to radiation. Upon irradiation, the fullerene compound converts surrounding molecular oxygen to highly reactive oxygen radicals, including superoxide radicals, which in turn attack the tumor cells and inhibit their growth.

Details of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and from the claims.

DETAILED DESCRIPTION

This invention relates to use of a fullerene compound as a photo-sensitizer to inhibit the growth of benign or malignant tumor cells. The fullerene compound is a fullerene core substituted, optionally via a C_{1-50} linker, with 1-30 ionic groups. When photo-exicited, the fullerene compound converts oxygen molecules into singlet oxygen and then the related free radicals, such as superoxide free radicals. The free radicals subsequently cause damage to surrounding tumor cells and thereby inhibit the growth of the tumor cells (i.e., reducing the number and size of the tumor cells). The irradiation source can be laser or other lights, e.g., fluorescence or X-rays. The irradiation can be of a wavelength of 400-1000 nm and an energy intensity of 10-300 J/cm^2, and the irradiation time can be 10-200 minutes.

The fullerene compounds which can be used to practice the method of this invention include those described in U.S. Patent No. 5,994,410. They can be synthesized by methods well known in the art. For example, a sulfonate-alkyl-fullerene compound can be prepared by reacting a fullerene with a strong Lewis base (e.g., naphthalide) to produce an anionic fullerene intermediate. The intermediate then reacts with a cyclic sultone to produce sulfoalkylfullerene. See Chiang, et al., *Chem. Lett.* 1998, 465.

An amino-fullerene can be prepared directly by reacting a fullerene with an amine at ambient temperature for 2 days. See Hirsch, et al., *Angew. Chem. Int. Ed. Engl.* 1991, 30, 1309.

A carbonate-ended fullerene compound can be prepared by reacting an anionic fullerene intermediate, via naphthalide, with succinic anhydride or reacting a carbonate-ended alkylamine or arylamine with $C_{60}(NO_2)_6$ in the presence of a base, such as triethylamine, at 40°C for 5-16 hours. See Chiang, et al., *J. Chem. Soc., Perkin Trans. 1*, 1999, 31.

A phosphorate-ended fullerene compound can be prepared by reacting a phosphorate-ended alkylamine or arylamine with $C_{60}(NO_2)_6$ in the presence of a base, such as triethylamine, at 40°C for 5-16 hours. See Chiang, et al., *J. Chem. Soc., Perkin Trans. 1*, 1999, 31.

A sulfate-ended fullerene compound can be prepared by reacting a sulfate-ended alkylamine or arylamine with $C_{60}(NO_2)_6$ in the presence of a base, such as triethylamine, at 40°C for 5-16 hours. See Chiang, et al., *J. Chem. Soc., Perkin Trans. 1*, 1999, 31.

A suitable fullerene compound or its salt in a sufficient amount is formulated with a pharmaceutically acceptable carrier to form a pharmaceutical composition before being administered to a subject in need of treatment of a tumor. "A sufficient amount" refers to the amount of the compound which is required to confer therapeutic effect on the treated subject. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described by Freireich et al., Cancer Chemother. Rep., 1966, 50, 219. Body surface area may be approximately determined from height and weight of the patient. See, e.g., Scientific Tables, Geigy Pharmaceuticals, Ardley, N.Y., 1970, 537. Effective doses will also vary, as recognized by those skilled in the art, depending on the route of administration, the excipient usage, the distance of tumor from the skin surface, the source of the irradiation, and the optional co-usage with other therapeutic treatments including use of other anti-tumor compounds.

The pharmaceutical composition may be administered via a parenteral route, e.g., topically, intraperitoneally, and intravenously. Examples of parenteral dosage forms include an active compound dissolved in phosphate buffer solution (PBS), or admixed with any other pharmaceutically acceptable carrier. Solubilizing agents, such as cyclodextrins, or other solubilizing agents well known to those familiar with the art, can also be included in the pharmaceutical composition.

An *in vitro* inhibition assay can be used to preliminarily evaluate a fullerene compound's ability to inhibit the growth of tumor cells. For example, a fullerene compound solution can be added to a pre-incubated cell suspension. Subsequently, the cell suspension is irradiated with fluorescence light, followed by further incubation. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide is added to the cell suspension to react with mitochrondrial dehydrogenase to form formazon, which is extracted with dimethyl sulfoxide (DMSO). The DMSO extract solution is immediately used for optical measurement to determine the quantity of the formazon, which correlates with the quantity of dehydrogenase or the relative number of the living cells.

The fullerene compounds that have been preliminarily evaluated can be further tested to confirm their efficacy by an *in vivo* inhibition assay. See Chiang, et al., *Proc. Electrochem. Soc.*, 1999, *99-12*, 238-249. For example, a tumor-bearing mouse can be first administered a suitable fullerene compound in PBS close to the tumor site. The mouse is

then kept in the dark while the fullerene compound is circulated to the tumor site. After the hair on and around the tumor site is removed, the tumor site is irradiated with a laser beam or other light source. After the irradiation, the growth of the tumor in the mouse is examined at different intervals. The inhibitory effect is evaluated by measuring the mouse's average body weight and tumor volume. The mouse is euthanatized by carbon dioxide asphyxiation. The final body weight and organ weight of the treated mouse are measured. Blood samples are withdrawn for biochemistry and hematology analyses. All such data can be used to evaluate the efficacy of the fullerene compound to treat tumor.

Without further elaboration, it is believed that one skilled in the art, based on the description herein, can utilize the present invention to its fullest extent. All publications recited herein are hereby incorporated by reference in their entirety. The following specific examples, which describe synthesis and biological testing of one compound that can be used in the present invention, are therefore to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Example 1:

(1) Synthesis of hexa(sulfobutyl)fullerene (FC_4S)

A dimethoxyethane (DME) solution of sodium naphthalide was first titrated with succinic acid. C_{60} was then treated with the sodium naphthalide DME solution (10.0 equiv.) at 25°C to produce a hexaanionic fullerene intermediate. The fullerene intermediate subsequently reacted with an excess of 1,4-butane sultone (15.0 equiv.) to produce FC_4S. It was obtained in a yield of 80-85% after purification by filtration and repeated precipitation in methanol from an aqueous solution. FC_4S gave a simple peak in its HPLC chromatogram using a reverse-phase C-18 column and H_2O as the eluent. Infrared spectrum of FC_4S displayed a broad absorption band centered at 3444 cm^{-1} (owing to a hydrated molecule), along with two strong absorption bands centered at 1178 and 1050 cm^{-1}, corresponding to the stretching bands of the sulfonate with a C-SO_2-O linkage. ^1H NMR spectrum of CF_4S in D_2O showed two broad peaks centered at δ 1.92 and 3.10, corresponding to the chemical shifts of -CH_2-C and -CH_2S protons, respectively.

Acidification of FC_4S with 4N HCl afforded the corresponding hexasulfonic acid, $C_{60}(CH_2CH_2CH_2CH_2SO_3H)_6$. A conceivable molecular ion of $C_{60}(CH_2CH_2CH_2CH_2SO_3H)_6$

was detected at m/z 1542 in the negative ion matrix-assisted laser desorption ionization (MALDI) mass spectrum. It was followed by two groups of ion fragments with peaks of a maximal intensity at m/z 1405 and 1269, corresponding to the masses of $C_{60}(CH_2CH_2CH_2CH_2SO_3H)_5$ and $C_{60}(CH_2CH_2CH_2CH_2SO_3H)_4$, respectively.

These data confirmed the structure and the composition of FC_4S.

(2) Irradiation-induced superoxide generation by FC_4S

The ability of generating superoxide free radical by FC_4S was demonstrated as follows: FC_4S aqueous solutions (1.0 ml each) at various concentrations (0-100 μM) were respectively added to ferricytochrome *c*-containing PBS (1.0 ml, 100 μM). Each of the mixtures was then added into a well of a 24-well plate and then exposed to fluorescence light (27 W) for 0-90 min. The distance between the plate cover and the light source was 5-6 cm. The extent of reduction of ferricytochrome *c* to ferrocytochrome *c* was evaluated by optical measurement. The increase of the absorbance at 550 nm corresponded to the increase of the quantity of ferrocytochrome *c*. Production of ferrocytochrome *c* indicated that FC_4S, upon irradiation, converted molecular oxygen to superoxide free radicals, and electron transfer from the superoxide free radicals to ferricytochrome *c* reduced the ferricytochrome *c* to ferrocytochrome *c*. It was observed that more superoxide free radicals were generated when the FC_4S dosage and the irradiation time were increased.

(3) *In vitro* irradiation-induced cytotoxicity of FC_4S based on tumor cell viability

Tumor cells were prepared as follows: Fibrosarcoma cells (CCRC 60037) and sarcoma 180 cells (obtained form Biochemical Institute of Chung Shan Medical and Dental College, Taiwan) were maintained and cultured in α-modified eagle medium (MEM) containing *L*-glutamine and phenol red, 10% fetal bovine serum, and antibiotics (100 units/ml of penicillin G and 100 μg/ml streptomycin sulfate). The cells were incubated in the dark in 95% humidified air plus 5% CO_2. After harvest by treatment with trypsin-EDTA, the cells were suspended in α-MEM medium at the concentration of 1×10^4 cells/ml.

Cell suspensions (500 μl each) thus obtained was placed into the wells of a 24-well plate and pre-incubated at 37°C for 24 hours. FC_4S solutions (500 μl each) at various concentrations (0-20 μM) were added to the wells. Each of the cell suspensions was irradiated with fluorescence light (27 watts) for 0-60 minutes. The distance between the plate cover and the light source was 5-6 cm. After the irradiation, the cells were further

incubated for 48 hours. A solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT, 0.5% in PBS, 100 µl each) was then added to each of the cell suspensions to react with mitochrondrial dehydrogenase enzyme in the living cells to produce formazon. The cell suspensions were again incubated for 3 hours at 37°C before the suspension medium was discarded. Formazon was then extracted using dimethyl sulfoxide (DMSO, 1.0 ml each). The DMSO extract solutions were immediately used for optical measurement. The absorbance at 540 nm directly correlated with the quantity of formazon, and thus with the quantity of dehydrogenase enzyme or the relative number of the living cells (i.e., viability). The results showed a decrease in cell viability with the increase in FC_4S dosage and irradiation time.

(4) *In vitro* irradiation-induced cytotoxicity of FC_4S based on tumor cell morphology

Fibrosarcoma cell (CCRC 60037) suspensions (4.0 ml) on glass coverslips were incubated in a 6-well plate. The suspensions were irradiation with fluorescence (27 watts) for 40 minutes in the presence of FC_4S at various concentrations (i.e., 2.5 - 5.0 µM). After further incubation for 48 hours, they were fixed with freshly prepared glutaraldehyde in PBS (2.5%) at 4°C for 2 hours. After the fixation, the coverslips were washed three times with PBS. The cells were then permeabilized by using methanol for 7 minutes. After removal of all liquid by air drying, the fibrosarcoma cells contained in the coverslips were stained with Giemsa stain solution (20× diluted) for 60 minutes and then transferred onto slides, each containing 10 µl mounting media, for examination with an optical microscope (Zeiss). A largely deteriorated cell structure with broken cell membrane of each cell was observed. The results showed excellent irradiation-induced cytotoxicity on the fibrosarcoma cells by FC_4S at a dose higher than 2.5 µM. At the dosage of 5.0 µM, all the cells were reduced to fragmented solid residues.

(5) *In vivo* irradiation therapy study of FC_4S

Irradiation therapy tests were conducted in male ICR mice (Charles River Japan origin Crl: CD-1®(ICR)BR), which were 8 weeks old and weighed 37±0.8 g. The mice were housed in polycarbonated shoe-box cages on hardwood bedding (5 mice/cage) under controlled pathogen-free conditions (temperature 22±1 °C, relative humidity 55±15%, and light/dark cycle 12/12 hours), and were allowed free access to a laboratory rodent diet (# 5K55, Purina Mills, Inc., St. Louis, MO) and water.

Murine sarcoma 180 cells were maintained in the abdominal cavities of other mice. Subcutaneous tumor was induced by intraperitoneal injection of 1×10^7 tumor cells (about 0.1-0.15 ml ascitic fluid) to the subcutaneous region of the abdominal cavity of each mouse. The tumor cells were allowed to proliferate at the inoculation site for 5-7 days. Fifty mice, each bearing a subcutaneous tumor in a diameter of 10±2 mm, were divided into 5 groups, i.e., 1. tumor control (without treatment); 2. intraperitoneal injection of FC_4S (15 mg/kg) without laser irradiation; 3. intraperitoneal injection of FC_4S (5.0 mg/kg) followed by laser irradiation; 4. intraperitoneal injection of FC_4S (10 mg/kg) followed by laser irradiation; and 5. intraperitoneal injection of FC_4S (15 mg/kg) followed by laser irradiation. Group 6, a tumor-free control group, was also used in the study.

After intraperitoneal injection of FC_4S in PBS (5-15 mg/kg body weight of mouse) 2.0 cm away from the tumor site, the mice were kept in the dark for 24 hours. The hair on and around the tumor sites was removed after the mice were anesthetized with avertine (0.3 ml/head). The tumor sites were each subsequently irradiated with an argon ion laser beam (Spectra Physics, Model 168) at a wavelength of 514.5 nm for 0-60 minutes. The beam was delivered via a quartz fiber with the circular area of illumination output focused to a diameter of 7-8 mm with the total light dose adjusted to a level of 100 J/cm^2 in each experiment.

After the treatment, the mice were examined every 5 days for 30 days. Efficacy of the irradiation therapy was evaluated by measuring the average body weight and tumor volume of each mouse. After 30 days, the mice were euthanatized with carbon dioxide asphyxiation. The final body weight and weights of organs, including liver, kidney, spleen, heart, and tumor, were measured. Blood samples were withdrawn, and plasma biochemistry and blood hematology analyses were conducted with a Hitachi 7050 Automatic Analyzer and a Serono System 9000, respectively.

The irradiation therapy-treated mice (groups 3, 4, and 5) showed a body growth rate close to that of tumor-free control (group 6). The untreated tumor-bearing mice in the control (group 1) had a lower body growth rate than that of mice in groups 2, 3, 4, 5, and 6.

The average weight of the tumor isolated from the irradiation therapy-treated mice (groups 3, 4, and 5) decreased with the increase in the FC_4S dosage. At the FC_4S dosage of 5.0 mg/kg (group 3), the tumor weight was 20% of that of the control (group 1) at day 30. At dosages of 10 and 15 mg/kg (groups 4 and 5), the average tumor weight was 10% of that of

the control (group 1). These results indicated high efficacy of FC_4S to reduce the viability of fibrosarcoma tumors in radiation therapy. Interestingly, FC_4S alone (group 2) was found to inhibit tumor growth by about 23%.

Analysis of blood samples showed differences in biological activities between treated and untreated mice. For example, after the radiation therapy, the mice in groups 3, 4, and 5 exhibited higher activities of asparate aminotransferase, alanine aminotransferase, and alkaline phosphatase than those in group 1. Lactate dehydrogenase activity was also higher in the mice in group 3, 4, and 5 than those in group 1 at day 30. These observations suggest damage to the tumor cell membrane and leakage of enzyme proteins. The levels of cholesterol and glucose were significantly higher in the mice in groups 3, 4, and 5 than those in group 1, indicating continued proliferation of tumor cells in the mice of group 1 enhanced consumption of these substrates.

Untreated tumor cells (group 1) proliferated continuously at a high rate as expected. The growth rates of the tumor cells in the mice in groups 3, 4, and 5 were much lower than group 1 in all testing periods. At day 30, the average tumor size of the mice in groups 3, 4, and 5 was 17% of that of those in group 1.

Example 2:

In vivo radiation therapy with FC_4S was conducted in male ICR mice by following the same protocol described in Example 1, except that irradiation with laser of the wavelength of 633 nm and intravenous administration were also employed.

The mice were divided into 5 groups: (1) tumor control (without treatment); (2) intraperitoneal administration of FC_4S (15 mg/kg) with laser irradiation at 514.5 nm; (3) intraperitoneal administration of FC_4S (15 mg/kg) with laser irradiation at 633 nm; (4) intravenous administration (tail) of FC_4S (15 mg/kg) with laser irradiation at 514.5 nm; and (5) intravenous administration (tail) of FC_4S (15 mg/kg) with laser irradiation at 633 nm.

Growth of tumor in these mice was measured at different time intervals (i.e., 5, 10, 15, 20, 25, and 30 days) after irradiation. The data showed that the inhibition on tumor growth by FC_4S was more effective when using the higher energy 514.5 nm laser than the lower energy 633 nm laser. Intraperitoneal injection of FC_4S resulted in only slightly better

inhibitory effect than the intravenous administration. The tumor cells were found to be completely eliminated at the dosage of 15 mg/kg by intraperitoneal administration.

Other Embodiments

A number of embodiments of the invention have been described above. It will be understood that various modifications may be made without departing from the spirit and scope of the invention. For example, the fullerene compounds used to practice the method of this invention include those which, in addition to being substituted with ionic groups, are further substituted with non-ionic groups (e.g., hydroxyl). Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of inhibiting the growth of tumor cells, comprising administering to a tumor site of a subject in need thereof a compound in an amount sufficient to inhibit the growth of the tumor cells at the tumor site, and, subsequently, exposing the tumor site to irradiation, wherein the compound is a fullerene core substituted, optionally via a C_{1-50} linker, with 1-30 ionic groups.
2. The method of claim 1, wherein the fullerene core is substituted via a C_{1-30} linker with 1-30 ionic groups.
3. The method of claim 2, wherein the fullerene core is substituted with 2-20 ionic groups.
4. The method of claim 2, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.
5. The method of claim 3, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.
6. The method of claim 5, wherein each of the linkers is alkyl, and each of the ionic groups, independently, is sulfonate, sulfate, carbonate, phosphonate, phosphorate, or ammonium.
7. The method of claim 6, wherein the fullerene core is substituted with 6 sulfonate groups.
8. The method of claim 1, wherein the fullerene core is substituted via a C_{2-16} linker with 1-30 ionic groups.
9. The method of claim 8, wherein the fullerene core is substituted with 2-20 ionic groups.
10. The method of claim 9, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.
11. The method of claim 9, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.
12. The method of claim 11, wherein each of the linkers is alkyl, and each of the ionic groups, independently, is sulfonate, sulfate, carbonate, phosphonate, phosphorate, or ammonium.

13. The method of claim 12, wherein the fullerene core is substituted with 6 sulfonate groups.

14. The method of claim 1, wherein the fullerene core is substituted via a C_{3-8} linker with 1-30 ionic groups.

15. The method of claim 14, wherein the fullerene core is substituted with 2-20 ionic groups.

16. The method of claim 14, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.

17. The method of claim 15, wherein each of the linkers, independently, is alkyl, aryl, ester, ether, thioether, urethane, urea, amide, anhydride, amine, or ketoether.

18. The method of claim 17, wherein each of the linkers is alkyl, and each of the ionic groups, independently, is sulfonate, sulfate, carbonate, phosphonate, phosphorate, or ammonium.

19. The method of claim 18, wherein the fullerene core is substituted with 6 sulfonate groups.

20. The method of claim 19, wherein each of the linkers is C_4 alkyl.

21. The method of claim 1, wherein the fullerene core is substituted, optionally via a C_{1-50} linker, with 2-20 ionic groups.

22. The method of claim 21, wherein the fullerene core is substituted, optionally via a C_{1-50} linker, with 4-10 ionic groups.

23. The method of claim 22, wherein the fullerene core is substituted, via a C_{2-16} linker, with 4-10 ionic groups.

24. The method of claim 22, wherein the fullerene core is substituted, via a C_{3-8} linker, with 4-10 ionic groups.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/29081

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A61N 1/30
US CL : 604/20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 604/19, 20, 27, 28, 46, 48, 500-502, 506-512, 514, 518-522, 290

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
BRS: tumor, irradiation, fullerene

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	US 6,162,926 A (Murphy et al.) 19 December 2000, see entire patent and usage with tumors.	1-24
Y	US 5,994,410 A (Chiang et al.) 30 November 1999, see entire patent and fullerene compounds.	1-24
A	US 5,648,523 A (Chiang) 15 July 1997, see entire patent.	1-24
A	US 5,635,581 A (Chiang et al.) 03 June 1997, see fullerene compounds.	1-24
A	US 5,416,188 A (Chiang et al.) 16 May 1995, see fullerene compounds.	1-24
A	US 5,294,732 A (Chiang et al.) 15 March 1994, see fullerene compounds and uses.	1-24

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Michael Thompson Telephone No. (703) 308-0858

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